# Establishing a *MITO-TagAd-GFP* Mouse Colony to Study Maternal Obesity

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## Introduction

Obesity is a global threat. Previous studies determined that maternal obesity increases the likelihood of obese offspring with lifelong complications. Specifically, prior to implantation, the early embryonic period is susceptible to the effects of obesity, which causes proinflammatory adipokines to promote white adipose tissue (WAT) dysfunction. Through oxidative stress in WAT, it is proposed that ruptured-mitochondria release mitochondrial DNA (mtDNA) that is harbored in extracellular vesicles (EVs). Being crucial communication molecules, EVs carry cargo such as mitochondria or mtDNA to distal sites and can induce inflammation and activate an immune response. Thus, we hypothesize that maternal obesity causes WAT to release EVs that contain mtDNA or mitochondria that then migrate to the preimplantation embryo and induce abnormal metabolic function in the offspring. In order to follow mitochondrial migration in EVs from maternal adipose tissue, our group is crossing several transgenic murine lines with the goal of producing a mouse that has red fluorescent mitochondria in green fluorescent-tagged EVs from adipocytes. This model will enable in vivo and in vitro tracking of adipocyte EVs via fluorescent microscopy.

The overarching purpose of this study is to determine if maternal adipocyte EVs transfer mitochondrial components to the embryo and alters the metabolic homeostasis of the embryo and offspring.

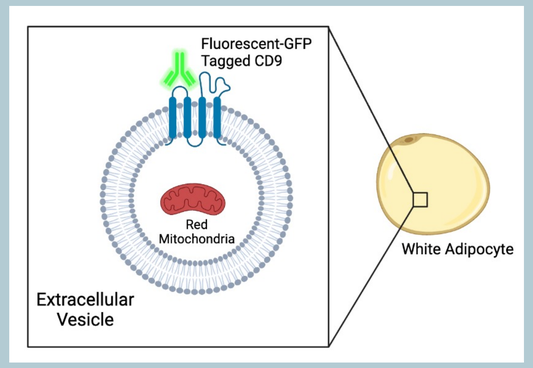


**Figure 1.** Schematic of red fluorescent mitochondria in green fluorescent-tagged EVs from adipocytes

## Focus

The focus of my project is to genotype mice from the five original colonies as well as the crossed colonies.

## Methodology

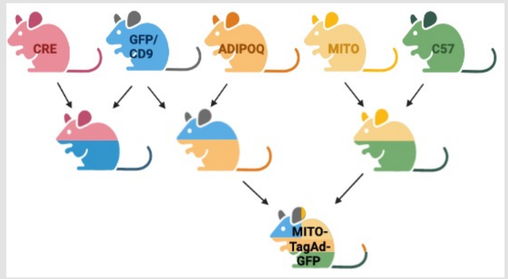


**Figure 2.** Mating plan for *MITO-TagAd-GFP* murine colony.

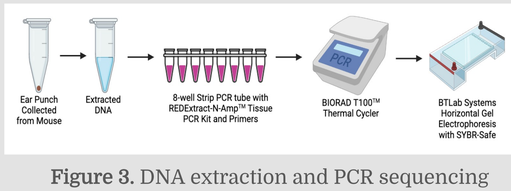


**Figure 3.** DNA extraction and PCR sequencing

| Name | Primer sequence (5' → 3') |
|---|---|
| C57BL/6J WT F | GTA GGG CCA ACT GTT TCT GCA TGA |
| C57BL/6J WT R | GGG CAT AGG AAG CAA ATA CCA AGT TG |
| AdipoQ WT F | CCG CAT CTT CTT GTG CAG T |
| AdipoQ WT R | ATC ACG TCC TCC ATC ATC C |
| AdipoQ MT F | GAG TCT GCC TTT CCC ATG AC |
| AdipoQ MT R | TCC CTC ACA TCC TCA GGT TC |
| mKate2 WT F | AGT GGC CTC TTC CAG AAA TG |
| mKate2 WT R | TGC GAC TGT GTC TGA TTT CC |
| mKate2 MT F | GCC AAG ATC CAT TCG TTG |
| mKate2 MT R | CCT TGA TTC TCA TGG TCT GG |
| CD9/GFP WT F | CTG GCT TCT GAG GAC CG |
| CD9/GFP WT R | AAT CTG TGG GAA GTC TTG TCC |
| CD9/GFP MT F | TGC CGT GGT CAT GAT ATT TG |
| CD9/GFP MT R | ATG CGG CAC TCG ATC TCC |
| cre/ERT2 WT F | CTG GCT TCT GAG GAC CG |
| cre/ERT2 WT R | CCG AAA ATC TGT GGG AAG TC |

**Figure 4.** Primer sequences. WT=wildtype. MT=mutant

- Mice were ear-tagged, and ear punches were collected on day 28.
- Mice are harem-mated with two females and one male at 8 weeks old.
- Cre + GFP/CD9, AdipoQ + GFP/CD9, mito::mKate2 (mito) + Cre, mito + C57 have been crossed (Figure 2).
- SYBR-Safe PCR was performed on extracted DNA of murine ear punches from CD9/GFP, cre/ERT2, mito, AdipoQ, and C57BL/6 strains. This was enough to determine zygosity for CD9/GFP, cre/ERT2, mito, and C57BL/6 mice (Figure 3).

## Future Directions

- Determine zygosity of AdipoQ mice using Taq-Man qRT-PCR.
- Compare the concentration/content of EVs.
- Determine the functional effects of EVs.
- Determine viability of embryos.
- Determine EV localization in embryos.
- Compare pup count from obese and lean mice.
- Measure the concentration of hormones.
- Analyze mitochondrial function.

## Acknowledgements

I would like to thank Lindsey Block, Erin Morris, and Dr. Rebecca Simmons from the Center for Research on Reproduction and Women's Health at Perelman Medicine for their mentorship of the research project.

## Results

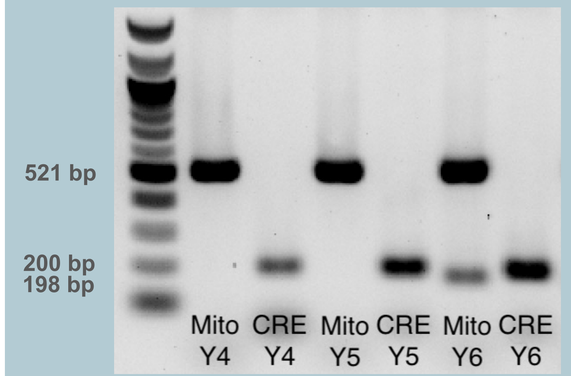


**Figure 5.** Representative Gel on PCR products from 3 mito+ Cre mice. WT mito= 521 bp. MT mito = 198 bp. WT Cre = 200 bp

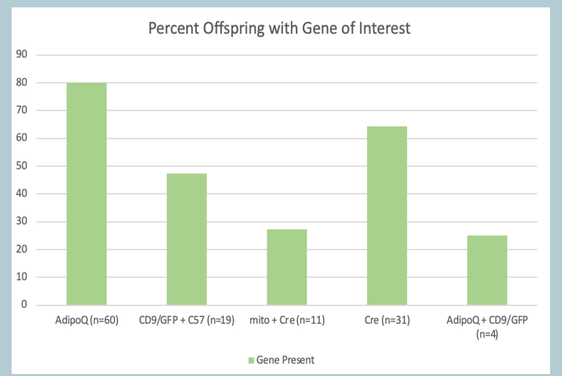


**Figure 6.** Percent offspring with gene of interest. (n=number of mice )

- 1 of 3 (Y6) mice were positive for the mito::mKate2 mutation (Figure 5).
- Figure 6 depicts the success rate of the crosses. These data support the successful crossing of these lines together.